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Short communication

LABORATORY EVALUATION OF THE TOXICITY OF METHANOLIC EXTRACT OF AFRICAN BUSH TEA SEED (*Hyptis suaveolens* Poit.) FOR THE CONTROL Of COWPEA BEETLE (*Callosobruchus maculatus* Fabricius).

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ABSTRACT

Powdered seeds of Hyptis suaveolens Poit. (Labiatae) were extracted with methanol at 64-65^0 C in Soxhlet extraction unit. The extract was placed over a water bath at 60^{0}C to evaporate the methanol which was used as solvent. Newly emerged adults of Callosobruchus maculatus F. (about 2-day old) were used in the evaluation of the toxicity of this extract. Different concentrations obtained by diluting the extract with acetone were prepared for use. The experiment was conducted in the laboratory at 25 $\pm$ 2^0 C and 70 $\pm$ 5% relative humidity. At all concentrations the extract had significant effect ($P < 0.05$) on C. maculatus compared to the extract-free control (acetone alone).

Key words: *Callosobruchus maculatus,* evaluation, *Hyptis suaveolens,* methanolic extract.

INTRODUCTION

Cowpea (*Vigna unguiculata* (L.) Walp) is a very important legume world wide and is grown in almost all the geo-ecological zones of Nigeria (Alabi and Emechebe, 2006). It is a widely distributed legume in the tropics especially Central and West Africa, which account for over 64% of the cultivated area (Singh *et al.,* 1997). Cowpea is a good source of proteins, complex carbohydrates, vitamins and minerals (Reyes – Moreno and Peredez – Lopez, 1993) but poor in methionine and cystine. In the year 2000, Nigeria produced about 2.1 million tonnes of cowpea out of the total world production of 3.3 million tonnes. This makes Nigeria the World's largest producer of cowpea (IITA, 2001). One of the major constraints confronting cowpea in storage is insect infestation most especially *Callosobruchus maculatus* (F.). The larvae and adults of this insect pest constitute the destructive stages attacking cowpea in storage. Severely damaged seeds are turned into powdery mass which is unfit for human consumption (Ofuya and Dawodu, 2002).

Hyptis suaveolens Poit. belongs to the family Labiatae (Lamiacae). It is called "Okwekwe" by the Igede people of Benue and Cross River States of Nigeria (Igoli *et al.,* 2003). *H. suaveolens* is between 0.2 and 1.0 m tall and has remarkable odour, which has a repelling effect on insects (Oparaeke *et al.,* 2002). The seeds are tiny and dark brown in colour. It is an age old practice to protect cowpeas with the pungent smelling inflorescence of *Hyptis spicigera* Lam. (Bugundu, 1970; Giles, 1964). The use of dried twig (Ajayi *et al.,* 1987), dried ground shoot (Fatope *et al.,* 1995) and leaf powder (Lajide *et al.,* 1998) of *H. suaveolens* Poit. against stored product insect pests is also well know. This is quite different from the use of seed extract where there may be some active ingredient effect. Early work has reported that the African bush tea, *H. suaveolens,* possesses certain alkaloids, acids and phenols (Dukes, 1980; Rao *et al.,* 1990).

The exorbitant cost of conventional insecticides and the problems of insecticide resistance and environmental unfriendliness make it expedient to consider the potential of this locally available plant and its product. This work examines the toxicity of the African bush tea seed extract as a cowpea beetle biopesticide.

MATERIALS AND METHODS

The *C. maculatus* used was obtained from naturally infested cowpea maintained in the Crop Protection Laboratory of the University of Ilorin, Nigeria. The culture was raised in a 250 ml Kilner jar at prevailing temperature and relative humidity (25 $\pm$ 5^{0}C and 70 $\pm$ 5%).

Table 1. Mean oviposition and adult emergence of *Callosobruchus maculatus* exposed to different concentrations of *Hyptis suaveolens* seed extract and acetone (control).

Concentrations of extract in acetone (mg/ml)	Egg count ± S.E. **	Adult count ± S.E. **
25	7.0 ± 1.15	67.0 ± 11.58
50	4.5 ± 0.58	27.2 ± 3.25
75	2.5 ± 0.58	18.8 ± 3.80
100	1.5 ± 0.58	9.2 ± 0.91
Acetone (control)	49.5 ± 1.29	80.0 ± 7.87
Mean	13.0	40.4
LSD (0.05)	1.34	10.03
CV (%)	6.9	16.5

** Values represent grand means of four replicates.
LSD = Least Significant Difference.
SE = Standard error
CV = Coefficient of Variation
DAI = Days after infestation

Table 2: Mean mortality of *Callosobruchus maculatus* exposed to different concentrations of *Hyptis suaveolens* seed extract and acetone (control).

Concentrations of extract in acetone (mg/ml)	Mean Adult Mortality ± SE Days after treatment *		
	1	2	3
25	0.5 ± 0.58	0.8 ± 0.54	1.0 ± 0.82
50	1.2 ± 0.54	1.2 ± 0.54	1.5 ± 0.29
75	1.2 ± 0.54	1.8 ± 0.91	2.2 ± 0.40
100	1.5 ± 1.29	23.8 ± 0.40	3.5 ± 3.00
Acetone (control)	0.0 ± 0.00	0.2 ± 0.16	0.50 ± 0.58
Mean	0.88	1.36	1.74
LSD (0.05)	1.11	0.88	2.36

*Values are means of four replicates.
LSD = Least Significant Difference
SE = Standard error

Oil extraction: Seeds of the *H. suaveolens* were obtained from an uncultivated land in Ilorin, Nigeria and dried in an oven at 80^{0}C for 4 hours. The seed sample was then ground in a mortar using a pestle, filtered through a 40-mesh screen and extracted for 7 hours using the Soxhlet apparatus (A.O.A.C. 1970) with methanol b.p 64-65^{0}C. The extract was placed in an open 250 ml conical flask in the water bath at 60^{0}C for 4 hours to evaporate the methanol. The final product was an oily yellowish crude extract.

Experimental procedure: The pure extract was then made to different concentrations of 25, 50, 75 and 100 mg/ml with pure acetone in screw-capped bottles. Transparent containers (9 cm in diameter) were each lined with Whatman No. 1 filter paper on which 5 drops (using pipette) of the different concentrations of the extract were applied. The filter papers were dried for one hour before the release of 50 g cowpea seeds and beetle adults on them. Five newly emerged adults (2 males and 3 females) of *C. maculatus* (about 2–day old) were then placed in each container and covered with muslin cloth. An extract-free control (acetone only) was set up and all treatments were replicated four times. Counts of the number of eggs laid per 20 cowpea seeds were examined ten days after infestation (DAI) and the introduced adults removed and discarded to prevent overlap with the first filial generation. The total number of adult emergence 30 DAI in each treatment was counted and recorded. Separate but similar experimental set up was prepared for determination of adult *C. maculatus* mortality. Counts of dead seed beetles were made after decanting the content of each container on the laboratory desk. The seed beetles were considered dead if they remained stiff after they had been pricked with insect pin. The surviving beetles were returned to the containers while the dead beetles were counted and discarded. Mortality counts were made on daily basis for three days. Data collected were subjected to analysis of variance (ANOVA) and Least Significant Difference (LSD) was used to partition the means.

RESULTS AND DISCUSSION

Table 1 shows the effect of the different concentrations of the methanolic extract of *H. suaveolens* seeds on oviposition and adult emergence of *C. maculatus*. At 25 mg/ml and 100 mg/ml concentrations, *C. maculatus* oviposited on the average 7.0 eggs and 1.5 eggs respectively while an average of 49.5 eggs were laid in the control. The egg counts were similar in the four concentrations but significantly lower ($P < 0.05$) than the control. It was observed that with lower dosage of 25 mg/ml and 50 mg/ml of cowpea, significant reduction in *C. maculatus* oviposition was achieved. The mode of action is partially attributed to interference with normal respiration resulting in suffocation (Hall and Harman, 1991). *C. maculatus* eggs have a respiratory pore with a gas-exchange function which is blocked by plant oils (Credland, 1992; Daniel and Smith, 1994). There was beetle emergence 30 DAI in the concentrations tested (Table 1). Although 50 mg/ml, 75 mg/ml and 100 mg/ml concentrations produced significantly lower ($P < 0.05$) number of adult emergence than the control, the mean adult emergence recorded at 25 mg/ml did not significantly differ with the control. Mean adult emergence at 50 mg/ml, 75 mg/ml and 100 mg/ml were 27.2, 18.8 and 9.2 respectively (corresponding to a ratio of 3:2:1 approximately), and were significantly lower ($P < 0.05$) than 67.0 and 80.0 recorded in 25 mg/ml concentration and the extract-free control respectively. The toxicity of the *H. suaveolens* seed extract on eggs and adult emergence varies with concentrations. The active ingredients present in the test plant may be responsible for the observed reduction in number of eggs and adult emergence. The modes of action of the active ingredients probably blocked the respiratory pore of the eggs and toxic to the immature stages and adults of C. maculatus.

For the seed extract at concentrations of 25 mg/ml, 50 mg/ml and 75 mg/ml, the mean adult mortality of *C. maculatus* was not significantly different ($P > 0.05$) at two and three DAI (Table 2). The mean adult mortality was not significantly different among 25 mg/ml and 50 mg/ml concentrations and acetone alone (control) at two and three DAI. *H. suaveolens* seed extract was observed to cause significant mortality of adult beetles at 75 mg/ml and 100 mg/ml of cowpea compared to the control. The toxicity of *H. suaveolens* seed extract probably showed non-persistence with increase in period of exposure. It has been observed that active pesticidal principles of plant origin do not persist in the environment for a long time (Wink, 1993). This result corroborates the findings that *H. suaveolens* possesses insecticidal properties (Rao *et al.*, 1990). The active ingredients in *Hyptis* species have been reported as hydrobenzoic and hydrocinnamic acids (Schulz and Karl, 1980). The compounds present in *H. suaveolens* act by deterring insects from feeding, others kill on contact with the insects (Simmond and Blaney, 1992). Lately the insecticidal activities of petroleum ether extract of *H. suaveolens* seeds on second instar larvae of the Diamond back moth, *Plutella xylostella* (L.) was reported (Keita *et al.*,2006). However, Oparaeke *et al.* (2002) reported a less effectiveness of *H. suaveolens* leaf powders in its insecticidal action aginst *C. maculatus.*

CONCLUSION

From these results, it is concluded that *H. suaveolens* seed extract serves as toxicant against *C. maculatus* on stored cowpea. *H. suaveolens* has high toxicity and it is non-persistent. *H. suaveolens* is an herb found growing naturally on uncultivated lands thereby readily available for use by farmers for protection of small farm products against *C. maculatus*.

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